

*Although incidence of poliomyelitis in this relatively isolated community of western Montana has been considerably lower than in more densely populated urban areas, the epidemiological characteristics of the disease have remained unchanged.*

# Naturally Occurring Poliomyelitis Antibody in Hamilton, Montana

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SEROLOGIC SURVEYS have been useful for epidemiological study of many diseases, detection of their occurrences in communities, and determination of the immune status of populations to such diseases. They have been especially applicable to studies of poliomyelitis, and, for this particular disease, have been greatly enhanced by the development of simplified, tissue-culture tests for neutralizing antibody. Among the more extensive poliomyelitis investigations in the United States is the one of Melnick and his associates (1) made in Charleston, S. C., and Phoenix, Ariz. Fox and his associates (2) have thoroughly studied three communities in Louisiana.

The survey reported here was conducted in a rural community in western Montana. Data portraying incidence of poliomyelitis antibody in this area emphasize particularly the relationship between age and prevalence of antibody, relationship between age and antibody titer, and intrafamilial spread of poliovirus.

## General Procedure

The community studied was Hamilton, Mont., and its environs of approximately 24 square miles. Population of this area is about 5,500, of which 75 percent reside in the town itself. Mountainous terrain, practically without settle-

ment, extends 75 miles east, 100 miles south, and 125 miles west from the study area. U. S. Highway 93 passes through Hamilton in a north-south direction; a spur busline connects it with transcontinental transportation at Missoula, 50 miles north. In contrast to many densely populated Atlantic and Pacific coastal areas, this community is relatively isolated with respect to geographic setting and proximity to main commercial routes of travel.

Through cooperation of the four schools in the area, 1,034 blood samples were collected in February 1955 for serologic study. Most were from children in grades 1 through 12, but 138 were from adults and 50 were from preschool children. Specimens were obtained from 94 percent of the pupils in the three elementary schools and from 76 percent in the high school. Brief histories, including age and location of residence, were obtained from all participants. Presumably, none of them had received poliomyelitis vaccine, since commercial vaccine was

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not yet available and experimental vaccine had not been tested in this area. Serums were classified according to age of the donor (table 1); age 6-7, for example, signified any age from 72 months through the day preceding the eighth birthday. Children less than 6 years old were grouped as preschool children, and persons 21 years or older were grouped as adults.

### Laboratory Procedures

Serums were frozen at  $-24^{\circ}$  C. until tested for poliomyelitis neutralizing antibody. A metabolic inhibition test, similar to one described by Salk and co-workers (3), was employed. All serums were tested in twofold dilutions, 1:8 through 1:1,024. Serums with 1:8 or higher titer were designated seropositive. Those with titer of 1:1,024 might have been positive also in higher dilutions, if such dilutions had been tested.

### Age and Incidence

With a few exceptions, incidence of antibody to a specific type of poliovirus or to a combination of types gradually increased as age of the subjects increased (table 1). The greatest deviation from this trend occurred with respect to type 2 antibody, which was found in 48 percent of children aged 14-15, but in a considerably smaller percentage of children immediately older or younger.

There was no evidence that poliovirus either had recently entered the community or had been present several years before and then disappeared, leaving only older persons immune. In all age groups, type 2 antibody was more prevalent than type 3; in all except the 12-13 and 14-15 age groups, type 1 antibody was more prevalent than either type 2 or type 3. Among preschool children, 51 percent possessed one or more types of antibody, and the percentage gradually increased by age groups to 96 percent in adults. However, only 2 percent of preschool children, about 5 percent of high school students, and 20 percent of adults had all three types of antibody.

### Age and Antibody Titer

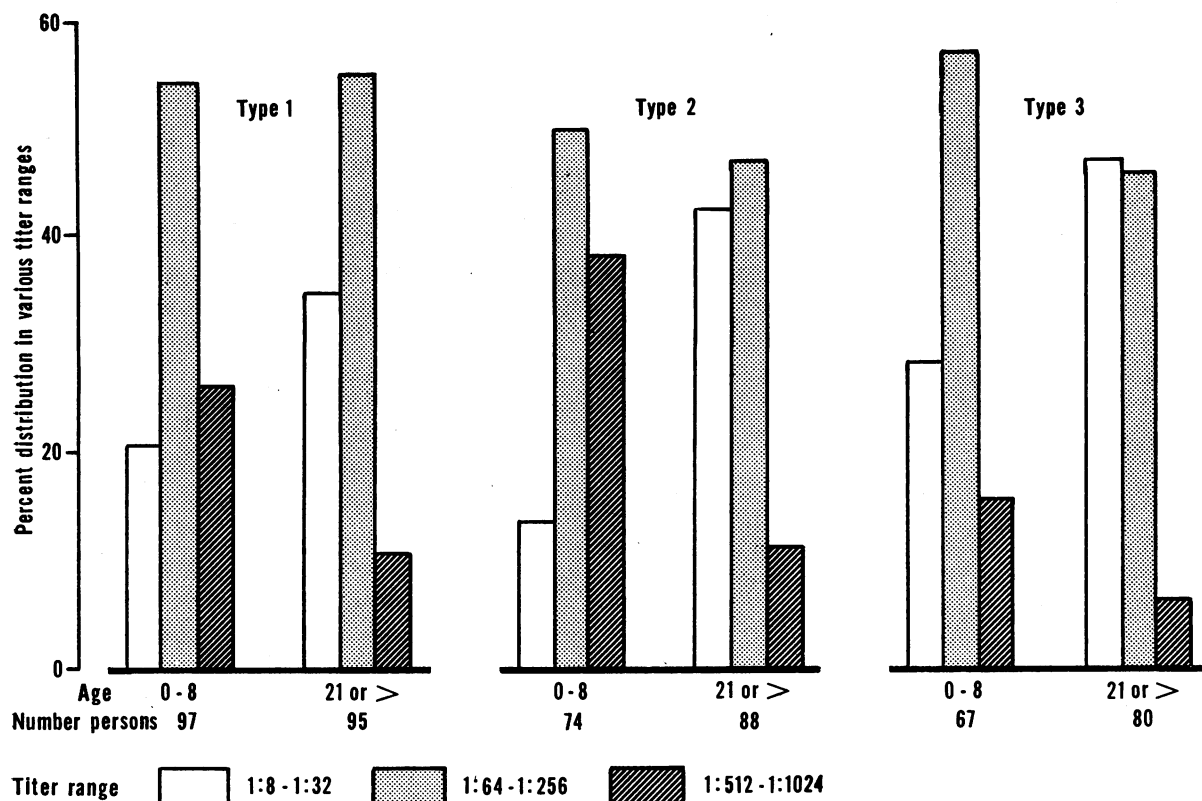
Seropositive serums from the adults and from children through 8 years of age were compared on a basis of antibody titers to the three polioviruses. This age range of children was selected so that numbers of seropositive serums from them and from the adult group would be similar. Type 1 antibody occurred in 97 of the 276 children and in 95 of the 138 adults (see chart). Whereas 21 percent of the children and 35 percent of the adults had titers in the 1:8 to 1:32 range, 26 percent and 10 percent, respectively, had titers in the 1:512 to 1:1,024 range. Almost equal percentages had titers in the intermediate range of 1:64 to 1:256. Titers of antibody to the other polioviruses in the two age groups were similarly

**Table 1. Distribution of poliomyelitis antibody in a population sample of 1,034 persons, Hamilton, Mont., by age group and poliovirus type**

Age (years)	Number of persons in group	Percent seropositive against—				
		Type 1	Type 2	Type 3	All 3 types	One or more types
Under 6 (preschool)-----	50	31	14	12	2	51
6-7-----	141	33	28	24	3	61
8-9-----	152	39	33	28	6	67
10-11-----	155	37	36	34	7	68
12-13-----	157	37	37	33	7	70
14-15-----	130	44	48	35	4	80
16-17-----	111	53	38	36	6	85
21 or over (adults)-----	138	69	64	58	20	96

<sup>1</sup> With reference to the chart, 85 children in this group were 8 years old.

**Relationship between age and poliomyelitis antibody titer in persons who were seropositive.**



compared. However, because the same type or types of antibody did not occur in all persons, the composition of the groups included in the second and third analyses varied somewhat from that of the first.

Regardless of antibody type, more high titers were found in seropositive children than in seropositive adults. Differences were greatest in the type 2 analysis where only 11 percent of adults had titers in the high range as compared with 38 percent of children.

#### **Intrafamilial Spread of Poliovirus**

Serums from all children were grouped according to families (table 2). The extent to which poliovirus spreads within a family following its introduction by one or more carriers was studied by examining the degree of similarity in seroimmune patterns of children within each family. Each of the three types of poliovirus was considered a distinct disease entity, capable of invading a family independently of the other two types. Each

family, therefore, represented three family-type combinations. If, for example, the two children in a particular family both possessed type 1 antibody, neither had type 2, and only one had type 3, two of the combinations were said to be in agreement, but the third, in disagreement.

Of the 468 combinations represented by families with two children, 87 percent were in agreement and 13 percent were in disagreement. The analysis was continued to determine how many instances of disagreement could be explained on the basis of age difference. If the child possessing antibody was the older, perhaps he had acquired the antibody through natural infection before the younger child was born. Sixty-six percent of the instances of disagreement could be accounted for in this manner. As a final figure, 96 percent of the combinations represented by families with two children gave either perfect agreement or disagreement which might be explained by order of birth. Comparable final figures for families with 3 or 4 children were 90 and 79 percent,

**Table 2. Common occurrence of poliomyelitis antibody in multiple children of individual families**

Children per family	Number of families	Number of family-type combinations <sup>1</sup>	Percent of family-type combinations—	
			With perfect agreement in antibody pattern <sup>2</sup>	With perfect agreement or explainable <sup>3</sup>
2-----	156	468	87	96
3-----	64	192	71	90
4-----	19	57	63	79
5-----	4	12	92	-----

<sup>1</sup> See text for explanation.

<sup>2</sup> Antibody of a specific type either present in all children or absent in all.

<sup>3</sup> Explainable on the basis of order of birth of multiple children in a family.

respectively. Only 4 families with 5 children were available for study, but 92 percent of the combinations represented by them gave perfect agreement.

### Complement Fixation Studies

Complement fixation tests on 225 serums, representing a sample of approximately 40 percent of the children in the age group 0-12, were performed by Dr. David B. Lackman of this laboratory. Unheated antigens were used throughout. The percentages of the group possessing types 1, 2, and 3 neutralizing antibody were, respectively, 44, 37, and 25, while those with complement-fixing antibody were 7, 16, and 12.

### Discussion

The three principal conclusions from these data, namely, that prevalence of poliomyelitis antibody in people of this community is directly related to their age, that children generally have higher antibody titers than adults, and that intrafamilial contact rapidly disseminates poliovirus, agree with conclusions of other investigators who have studied entirely different communities, most of which have been urban. During the past 18 years, type 1 has apparently been the most common type of poliovirus in this community, type 3 has been the least common,

and type 2 has been intermediate. This relationship in prevalence of the three types also exists in other areas of Montana (4). The high incidence of type 2 antibody in the 14-15 year age group, as compared with older or younger children, probably reflects past invasion of one particular classroom by type 2 virus.

Rate of acquisition of poliomyelitis antibody with increasing age appears lower in this community than in many other parts of the United States and is definitely lower than that in many other parts of the world. However, differences in methods of testing serums, including variations in the lowest serum dilution tested, complicate direct comparison of results reported by different laboratories. In the current investigation, only 6 percent of children 16-17 years old possessed all three types of antibody. Identical results have been reported from Swiss children aged 18-19 (5). In contrast, 40 percent of 5-year-old children in urban areas of Gothenburg, Sweden (6), and 75 percent of children 3 to 4 years old and more than 90 percent of children aged 7-9 in families of low socioeconomic class in Peru and Colombia (7) possess all three types of antibody. Poliovirus apparently spreads in people of western Montana at a rate similar to that in the higher socioeconomic class of people in Charleston, S. C., but at a much slower rate than in this class of Phoenix, Ariz., or in the lower socioeconomic class of either city (1). Poliovirus also spreads much more slowly in this Montana community than in three urban and semirural communities of Louisiana (2).

Slow dissemination of poliovirus in this area is probably due to an interplay of many environmental factors. The community is well separated from others. Many children go to and from school in private buses, but there are no public conveyances. Since there are no private schools, all children attend the same public schools. Streets are wide, homes are relatively far apart, and most homes have ample space for the occupants. Nearly all homes have indoor sanitary facilities. Although many families have private wells for water supply and nearby septic tanks for sewage disposal, necessity for such utilities is not restricted to any particular socioeconomic class. During many months of the year, human carriers of

poliovirus apparently are nonexistent in the community, as noted in an unpublished sewage study by C. L. Larson and his associates.

Because socioeconomic differences in the community are minimal and have little relationship to factors ordinarily considered of public health significance, the correlation between incidence of antibody and socioeconomic status was not thoroughly studied. The older north and newer south sections of the town of Hamilton were compared, however. In the south section, where the average family is generally considered to be somewhat above that of the north in income, cleanliness, and education, 41 percent of 217 children were without antibody to any type of poliovirus. In the north section, 20 percent of 192 children were in a similar condition.

Since high titers were found more frequently in children than in adults, they appear more likely to result from recent initial infections than from continued reexposure throughout life. Melnick and his associates (8) derived the same conclusion from complement fixation tests of human serums collected in Cairo, Egypt. Because poliomyelitis complement-fixing antibody, which is of relatively short duration in the blood, was confined almost entirely to childhood, re-infections in older persons were assumed to be rare. Le Bouvier (9) likewise concluded from data on Cairo residents that poliomyelitis antibody tends either to be absent from children or to exist in high titer, while in adults many medium or low titers are intermingled, due to gradual diminution of titers with age.

The data on seroimmune patterns of multiple children within the same families (table 2) augment other information (10,11) on the importance of intrafamilial contact in spread of poliovirus.

Since complement fixation tests were performed on serums from children of a rather narrow age span, results are of limited value. Ratios of percentage of children possessing neutralizing antibody to percentage possessing complement-fixing antibody were 6.3, 2.3, and 2.1 for types 1, 2, and 3 antibody, respectively. Because poliomyelitis complement-fixing antibody disappears much more rapidly than neu-

tralizing antibody, these data would indicate that types 2 and 3 poliovirus had invaded children 0-12 years old more recently than had type 1.

## Summary

Incidence of poliomyelitis antibody resulting from natural infection in a relatively isolated rural community of western Montana was determined by tests of 1,034 serums collected from residents, mostly children, in and near the town of Hamilton. Neutralizing antibody to all three types of poliovirus was present, but in nearly all age groups type 1 antibody was the most prevalent and type 3 the least prevalent. Incidence of antibody generally increased with increasing age of the population, but spread of poliovirus has been much slower in this community than in densely populated areas. Only 5 percent of the high school pupils, aged 14-17, had been infected by all three types of virus. High antibody titers occurred more frequently in children than in adults. Multiple children in individual families were, in most instances, either all immune or all non-immune to a particular type of virus, a fact which portrays the importance of household contact in spread of poliovirus through a community.

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## New Simplified Germ-Free Laboratory

The possibility that germ-free animals may be used for experiment in small laboratories of health departments and universities may be sped by the outcome of field tests of a device which has been demonstrated successfully at the University of Michigan. The cabinet was designed by Dr. Richard Horton, now with the National Institute of Allergy and Infectious Diseases of the Public Health Service.

Similar units are being made available to several institutions for field trials, some to be tested by experienced investigators and others to be tested by men with no previous experience with germ-free techniques.

Although germ-free laboratories have been operated successfully at Notre Dame University, Walter Reed Army Medical Center, and the National Institutes of Health, the cabinet designed by Dr. Horton is intended to func-

tion on a relatively simple and economical scale.

The apparatus, a sealed box of glass and metal, is about the size of a frozen food cabinet. Investigators reach into the sterile area through sealed-in rubber gauntlets. A pressure compartment at one end sterilizes all food and equipment by steam before it enters the living quarters of the germ-free animals. All air introduced into the cabinet is heated to 750° F. and is then cooled before it passes into the chamber. Excess food and feces have remained in the box for months without odor or signs of decomposition.

Dr. Gerald Abrams, Michigan pathologist, who tested the machine with guinea pigs delivered into the cabinet by sterile cesarean section, believes the device is also virus proof, but he has not yet tested that hypothesis.